The distribution of fibronectin and tenascin along migratory pathways of the neural crest in the trunk of amphibian embryos

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Summary

It is generally assumed that in amphibian embryos neural crest cells migrate dorsally, where they form the mesenchyme of the dorsal fin, laterally (between somites and epidermis), where they give rise to pigment cells, and ventromedially (between somites and neural tube), where they form the elements of the peripheral nervous system. While there is agreement about the crest migratory routes in the axolotl (Ambystoma mexicanum), different opinions exist about the lateral pathway in Xenopus. We investigated neural crest cell migration in Xenopus (stages 23, 32, 35/36 and 41) using the X. laevis–X. borealis nuclear marker system and could not find evidence for cells migrating laterally. We have also used immunohistochemistry to study the distribution of the extracellular matrix (ECM) glycoproteins fibronectin (FN) and tenascin (TN), which have been implicated in directing neural crest cells during their migrations in avian and mammalian embryos, in the neural crest migratory pathways of Xenopus and the axolotl. In premigratory stages of the crest, both in Xenopus (stage 22) and the axolotl (stage 25), FN was found subepidermally and in extracellular spaces around the neural tube, notochord and somites. The staining was particularly intense in the dorsal part of the embryo, but it was also present along the visceral and parietal layers of the lateral plate mesoderm. TN, in contrast, was found only in the anterior trunk mesoderm in Xenopus; in the axolotl, it was absent. During neural crest cell migration in Xenopus (stages 25–33) and the axolotl (stages 28–35), anti-FN stained the ECM throughout the embryo, whereas anti-TN staining was limited to dorsal regions. There it was particularly intense medially, i.e. in the dorsal fin, around the neural tube, notochord, dorsal aorta and at the medial surface of the somites (stage 35 in both species). During postmigratory stages in Xenopus (stage 40), anti-FN staining was less intense than anti-TN staining. In culture, axolotl neural crest cells spread differently on FN- and TN-coated substrata. On TN, the onset of cellular outgrowth was delayed for about 1 day, but after 3 days the extent of outgrowth was indistinguishable from cultures grown on FN. However, neural crest cells in 3-day-old cultures were much more flattened on FN than on TN. We conclude that both FN and TN are present in the ECM that lines the neural crest migratory pathways of amphibian embryos at the time when the neural crest cells are actively migrating. FN is present in the embryonic ECM before the onset of neural crest migration. In contrast, the appearance of TN is correlated with the initiation of migration. Since amphibian neural crest cells find FN, but not TN, to be adhesive migratory substrata in vitro, our results suggest that an interaction between these ECM components is important in regulating the onset and pathways of neural crest cell migration in the amphibian embryo.

Key words: Xenopus laevis, Ambystoma mexicanum, neural crest, extracellular matrix, fibronectin, tenascin, migratory pathway.

Introduction

Neural crest cells are the embryonic progenitors of the pigment cells, the neurones and glia of the peripheral nervous system and a variety of connective and endocrine tissues (for reviews see Weston, 1970; Noden, 1980; Le Douarin, 1982). In amphibian embryos, neural crest cells arise from the apical edges of the neural folds, which ultimately fuse and form the neural tube. From their position along the dorsal
surface of the neural tube, neural crest cells migrate either dorsally into the expanding dorsal fin, laterally between the somites and epidermis, or ventromedially between the neural tube and somites (Schroeder, 1970; Macmillan, 1976; Vogel & Model, 1977; Löfberg et al. 1980). The choice of pathway may be related to the composition of the extracellular matrix (ECM; Spieth & Keller, 1984; Löfberg et al. 1985, 1988; Tucker, 1986; Perris, 1987), which in amphibians includes chondroitin sulphate proteoglycan (CSPG), hyaluronate (Tucker, 1986; Tucker & Erickson, 1986b), collagen (Löfberg et al. 1980) and fibronectin (FN; Heasman et al. 1981). In avian embryos, FN has been shown to be necessary for normal neural crest cell migration (Boucaut et al. 1984; Bronner-Fraser, 1986) and, in amphibian embryos, FN is required for cell movements during gastrulation (Boucaut et al. 1984). Amphibian neural crest cells spread and migrate on FN-coated substrate, but they are unable to do so on substrata containing CSPG (Tucker & Erickson, 1986a; Perris & Johansson, 1987; Epperlein, 1988). This suggests that, as for avian neural crest cells, FN is necessary for normal neural crest cell migration and morphogenesis in amphibians.

The distribution of FN in avian and mammalian embryos is well documented (Newgreen & Thiery, 1980; Duband & Thiery, 1982; Thiery et al. 1982; Krotoski et al. 1986; Mackie et al. 1988). Although present in all neural crest pathways, the distribution of this molecule is too widespread for it to direct the migrations of neural crest cells alone. Recently, the distribution of tenascin (TN), another major glycoprotein of the embryonic ECM, has been described (Mackie et al. 1988). In quail and rat embryos, TN has a much more restricted distribution than FN, being limited to the anterior half of each somite, a major pathway region of trunk neural crest cells. This distribution suggests that TN, together with FN, may control pathways of neural crest cell dispersion in avian and mammalian embryos.

We have shown previously that both FN and TN are present in the trunk ECM of Xenopus laevis embryos (Mackie et al. 1988). In the current study, we have investigated the precise timing and migratory pathways of early neural crest migration in Xenopus using the X. laevis—X. borealis marking system (Thiebaud, 1983). This technique, which is based on the differential staining of X. laevis and X. borealis nuclei with the fluorescent dye quinacrine, permits the identification of grafted donor cells in a manner analogous to the quail—chick chimera system introduced by Le Douarin (1973). Then, using immunohistochemistry, we have undertaken a chronological survey of the distribution of both FN and TN in Xenopus and the urodele A. mexicanum (axolotl) before and during neural crest cell migration to determine if these ECM components may direct the timing of the onset or the pathways of neural crest migration in amphibians. Further, we have cultured amphibian neural crest cells on FN- and TN-coated substrata in order to determine if these molecules have a similar effect on neural crest cell morphology as has been reported for the avian neural crest (Mackie et al. 1988).

**Materials and methods**

**Embryos**

Wild-type (D−) embryos of Ambystoma mexicanum (the axolotl!) were obtained from the Department of Zoology, Uppsala, Sweden. They were staged according to the normal table of Bordzilovskaya & Dettlaff (1979). Xenopus laevis embryos had their origin from hormone-induced matings of animals maintained in colonies at Freiburg and Basel. X. borealis embryos were a gift from Dr R. Winklbauer, Max-Planck-Institut für Entwicklungsbiologie, Tübingen. The staging of X. laevis and X. borealis embryos was according to the normal table of Nieuwkoop & Faber (1975).  

**Antibodies**

Rabbit polyclonal antisera to human plasma fibronectin (BRL, Basel) were used. The rabbit polyclonal antiserum to chick tenascin were a gift from Drs R. Chiquet-Ehrismann and E. Mackie (Basel). The specificity of the anti-TN sera to chick TN has been reported previously (Chiquet-Ehrismann et al. 1986). The specificity of the antiserum against amphibian TN was determined by Western blotting. In brief, dorsal fins from stage-54 X. laevis were dissected free, boiled for 10 min in a small volume of x10 sample buffer, and loaded onto a 3-6–15 % gradient SDS–polyacrylamide gel adjacent to a 15 μg sample of immunopurified chick TN (a gift from Dr R. Chiquet-Ehrismann). The proteins were separated, transferred to a nitrocellulose filter (Towbin et al. 1979) and visualized with Ponceau red. The lane containing X. laevis proteins was cut in half: one strip was incubated in diluted TN antisera (1:100) and the other strip was incubated in similarly diluted preimmune serum following blocking in 5 % skim milk in phosphate-buffered saline (PBS). The strips were then rinsed and incubated in horseradish-peroxidase-conjugated goat anti-rabbit IgG. The antigens were visualized using 4-chloro-1-naphthol as chromogen.

**Fixation and sectioning**

X. laevis embryos at stages 22, 25, 28, 32, 35 and 40 and axolotl embryos at stages 18, 22, 25, 30, 35 and 40 were fixed in 4 % paraformaldehyde in 0·1 M-potassium phosphate buffer (pH 7·6) overnight at 4°C. The specimens were then rinsed in several changes of buffer and infiltrated with 5 % and 15 % sucrose in buffer, each overnight at 4°C. The tip of the tail was cut off from most embryos to improve the penetration of fixative and sucrose. Embryos were then embedded in OCT compound (Cambridge Instruments,
graphic records of the cultures were made with a Zeiss microscope. This method results in a uniform coating of the culture surface, as determined previously by staining with TN- and anti-TN antisera. For each substrate, two individual cultures were examined and at least 20 cells were measured.

**Immunochemistry**

Sections were blocked with 1% bovine serum albumin in 0.1 M-potassium phosphate buffer for 10 min, then incubated in diluted (1:100) polyclonal antisera to FN or TN for 1 h. Anti-FN was used to stain all of the stages indicated, whereas anti-TN was used to stain all sections except axolotl stages 18, 22 and 30. After rinsing in buffer, the sections were incubated in FITC-conjugated goat anti-rabbit IgG (Dianova, Hamburg) for 1 h. Sections were then rinsed and mounted in Hanks’ saline:glycerol (1:1). The sections were photographed using a Zeiss epifluorescence photomicroscope (BP 450-490, FT 510, LP 520) and Kodak Tri-X-Pan films.

**Results**

**Antibody specificity**

Since the specificity of the polyclonal anti-TN against amphibian TN has not been described previously, Western blots using larval X. laevis proteins were performed (Fig. 1). The anti-TN stains a band with an apparent relative molecular mass (Mr) of approximately 180x10^3 and a second broad band (characteristic of a glycosylated protein) at 200x10^3 from the crude Xenopus extract. The broad band has a similar mobility to the major band of purified chick tenascin, which was used as the source of antigen. The lower band found in the Xenopus extract may represent a proteolytic fragment or a slightly different form of Xenopus tenascin. Preimmune serum did not stain the Xenopus extract.

**Timing and routes of neural crest cell migration in Xenopus**

To determine the timing of the onset of migration as well as the distribution of neural crest cells during early development in Xenopus, the X. laevis-X. borealis labelling technique was used. The series of sections found in Fig. 2 documents the position of X. borealis (i.e. donor) neural crest cells in both transverse (Fig. 2A-C) and sagittal (Fig. 2D-F) sections. In operated embryos of stage 23 (very early tailbuds, 1 day 3-4 h old, according to Nieuwkoop & Faber, 1975), X. borealis neural crest cells are found in the wedge between dorsal somites and neural tube.
(Fig. 2A). At stage 32 (young tailbud, 1 day 16 h old) labelled neural crest cells are present on the ventromedial pathway, i.e. between somites and neural tube (Fig. 2B) or between somites and notochord or endoderm (Fig. 2C). Some labelled cells could be observed further ventrally in the vicinity of the kidney anlage. No borealis cells were found on the lateral route of neural crest cell migration, i.e. between somites and epidermis, nor could labelled cells be discovered within the somites. In embryos of stage 35 (2 day 2 h old), in which the eye is pigmented and a dorsal and a lateral melanophore stripe have formed, the internal distribution of labelled cells was similar to stage 32 (results not shown). At stage 41 (3 day 4 h old), a young larva about 1 day before feeding, many more labelled cells were present on the ventromedial pathway than in earlier stages. In Fig. 2D, which is a partial enlargement of the oblique sagittal section through the larva shown in Fig. 2F, several borealis cells can be recognized between the somites and notochord. In Fig. 1E, taken from a different stage 41 larva, a stream of borealis cells is observed at the ventral edge of the somites. At this stage, pigmented borealis cells are also observed within the lateral melanophore stripe (not shown). Thus, neural crest cells commence migration in X. laevis at approximately stage 23, and the primary pathway of neural crest migration in Xenopus appears to be ventrally, between the somites and neural tube/notochord. The latter observation is in agreement with the previous results of Macmillan (1976) and Tucker (1986).

Fibronectin and tenascin during neural crest cell migration

Sections through the anterior trunk region of X. laevis and axolotl embryos before the initiation of neural crest cell migration (i.e. stage 22 in Xenopus) (see above) and stage 25 in the axolotl [Löfberg et al. 1980]) were stained with antibodies to FN and TN. In the Xenopus embryo, anti-FN staining is found in the extracellular spaces separating each of the major blocks of tissues (Fig. 3A,B), i.e. around the neural tube, notochord and somites, as well as underlying the epidermis. Anti-FN staining is particularly intense in the dorsal part of the embryo, but it is also present laterally and ventrally, along the visceral and parietal layers of the lateral plate mesoderm. In contrast, anti-TN staining is limited to the lateral notochord and adjacent somitic mesoderm (Fig. 3C); no other staining was observed at this stage.

In the axolotl embryo, staining with anti-FN is similar to the staining observed in X. laevis; anti-FN staining is found in all of the major extracellular spaces (Fig. 4A,B). There is no staining with anti-TN (Fig. 4C) before neural crest migration.

Fibronectin and tenascin during trunk neural crest cell migration

In stage-25 to -33 Xenopus embryos, the neural crest cells migrate dorsally into the expanding dorsal fin or ventrally along the medial surface of the somites (see above). Pigment cells, which are derived from the neural crest and are visible through the translucent tissues, appear in the space between the somites and the endoderm and continue to migrate ventrally along the lateral plate mesoderm until stage 35, where they form the subepidermal lateral stripe (Tucker, 1986). Very few, if any, neural crest cells migrate laterally over the apices of the somites.

On frontal sections of stage-29/-30 Xenopus embryos, the differences between anti-FN (Fig. 5A, level of notochord; Fig. 5E, level of neural tube) and anti-TN staining (Fig. 5B, level of notochord; Fig. 5F, level of neural tube) during neural crest migration become particularly obvious. TN is present, like FN, in the intersomitic furrows but, unlike FN, TN is absent from subepidermal spaces. Anti-TN staining is much weaker around the neural tube and notochord than anti-FN staining and decreases in intensity posteriorly. This is further illustrated on accompanying transverse sections through embryos of the same age: Fig. 5C shows anti-FN, Fig. 5D anti-TN staining in the trunk.

During the late migration period (stage 35), anti-FN stains the ECM throughout the embryo including subepidermal spaces (Fig. 6A,B). The staining is especially intense in the dorsal fin, around the neural tube, notochord and the dorsal aorta, in the myosepta (intersomitic furrows) and ventrally between the endoderm and somites. With anti-TN, the staining in the dorsal part of the embryo (Fig. 6 C,D) resembles the staining with anti-FN. In contrast, there is no staining with antibodies to TN in midtrunk areas.
around the endoderm, i.e. in either layers of the lateral plate mesoderm.

In the axolotl, neural crest cells migrate laterally beneath the ectoderm as well as dorsally and ventrally (Löfberg et al. 1980). The migration commences at approximately stage 28-30. By stage 35, pigment cells are distributed ventrolaterally on the flank (Epperlein & Löfberg, 1984), while neurone precursors following the ventromedial pathway have reached the dorsal aorta (Vogel & Model, 1977). There is intense staining with anti-FN along each of the neural crest migratory routes at stage 35 (Fig. 7A,B). Anti-TN, in contrast, stains only areas in the medial portion of the embryo, i.e. the dorsal fin ECM, the medial surface of the somites and extracellular spaces around the neural tube, notochord, aorta and in the intersomitic furrows and spaces around bundles of myofibrils (Fig. 7C,D). Ventral to the dorsal aorta there is no staining with anti-TN.

Fibronectin and tenascin in the postneural crest migration larvae

The final migrations of neural-crest-derived cells that are readily traceable in the X. laevis larvae are the passage of melanophores from the ventral pathway through the somites and intersomitic furrows to the subectodermal space in the tail (Tucker, 1986). When the tails of stage-40 X. laevis larvae are stained with anti-TN antibodies, an intense reaction is observed throughout the ECM around the neural tube, notochord, aorta and in the intersomitic furrows and spaces around bundles of myofibrils (Fig. 8A,B). In contrast, the staining with anti-FN is only intense around the notochord and dorsal aorta, but is considerably less intense elsewhere in the embryo (Fig. 8C). There is little or no staining with anti-FN in the intersomitic furrows and fin matrices, though there is staining beneath the ectoderm and in the ECM surrounding myofibril bundles.

In the midtrunk at stage 40, the staining with both anti-TN and anti-FN is similar to the staining patterns found at stage 35 (results not shown).

Spreading and morphology of neural crest cells on FN- and TN-coated substrata

Axolotl neural fold explants grown on FN- or TN-
coated substrata show striking differences in spreading behaviour and neural crest cell morphology. On FN, crest cells begin to migrate from the explant after about half a day. At about 28 h of culture they have spread as a flattened sheet (Fig. 9A). On TN, no outgrowth is observed by 28 h (Fig. 9B), but spreading occurs in the same way as on FN following a 1 day delay. Once the neural crest cells have spread and migrate on TN-coated surfaces, their pattern of outgrowth resembles that of cultures grown on FN. However, the morphology of the cells is still different. In cultures of about 3 days, neural crest cells are more flattened on FN (Fig. 9C) than on TN (Fig. 9D). Calculations of the area taken by cells on either substrate support this notion. Cells on FN had a mean surface area of 2399 ± 704 µm², whereas cells cultured in parallel on TN had a mean surface area of 1249 ± 332 µm². Thus, the area occupied by neural crest cells on TN was reduced by 44% compared to cells on FN. Therefore, neural crest cells appear to find TN a less adhesive substratum than FN.

Discussion

Using the *X. laevis*–*X. borealis* nuclear marker system, we have shown that, in *Xenopus*, neural crest cells migrate ventrally, between the neural tube and somites, and not laterally, beneath the ectoderm. This is in agreement with a previous study using [³H]thymidine-labelled neural crest cells (Macmillan, 1976). Our results are in contrast to the claims of Krotoski & Bronner-Fraser (1986) and Sadaghiani & Thiebaud (1987), who concluded that the lateral pathway was an additional migratory route. The migration of *Xenopus* neural crest commences at approximately stage 23. In axolotl embryos, and in urodele embryos, in general, there is little disagreement about neural crest migratory pathways (see Raven, 1936; Detwiler, 1937; Chibon, 1967; Vogel & Model, 1977; Lofberg et al. 1980; Epperlein, 1982; Epperlein & Lofberg, 1984; Keller & Spieth, 1984). Neural crest cells are known to migrate dorsally where they form the mesenchyme of the expanding dorsal fin, laterally, where they give rise to pigment cells and ventromedially, where they form the

**Fig. 4.** Anti-fibronectin and anti-tenasin staining of transverse sections through the anterior trunk region of axolotl embryos before neural crest cell migration (stage 25). (A,B (enlarged)) As in *Xenopus*, anti-FN staining is found in all of the major extracellular spaces. (C) No staining was observed with anti-TN. Abbreviations as in Fig. 3. Bars, A: 200 µm; B: 50 µm; C: 200 µm.
elements of the peripheral nervous system.

We have used immunohistochemistry to determine if correlations exist between the commencement and migratory pathways of amphibian trunk neural crest migration with the initial appearance and distribution of two major components of the extracellular matrix, fibronectin and tenascin. Fibronectin is an important and perhaps essential substratum for cell migration during early amphibian development, including gastrulation (Boucaut et al. 1984) and primordial germ cell migration (Heasman et al. 1981). In avian embryos, FN is found throughout the ECM of the trunk, both before, during and after neural crest cell migration (Newgreen & Thiery, 1980; Duband & Thiery, 1982; Thiery et al. 1982; Krotoski et al. 1986). In the current study, we have observed a similar ubiquitous distribution of FN in amphibian embryos. Before neural crest cell migration, in both X. laevis and the axolotl, FN is found in the ECM throughout the embryo. During neural crest migration, FN is present both lining the migratory pathways as well as in areas where neural crest cells are absent. For example, X. laevis crest cells migrate primarily ventrally, between the somites and the neural tube, though anti-FN staining is present in both the ventral pathway as well as laterally, beneath the ectoderm.
Fig. 6. Anti-fibronectin and anti-tenascin staining of transverse sections through the midtrunk region of *X. laevis* during late neural crest cell migration (stage 35). (A,B (enlarged)) Anti-FN staining is found in the ECM throughout the embryo including subepidermal spaces. Staining is especially intense in the dorsal fin (df), around the neural tube (nt), notochord (not), dorsal aorta (da) and in the myosepta (ms). (C,D (enlarged, different embryo)) Anti-TN staining resembles the staining with anti-FN except for the entire ventral region, where no staining is observed. Bars, A,C: 100 μm; B,D: 50 μm.
Fig. 7. Anti-fibronectin and anti-tenascin staining of midtrunk transverse sections in the axolotl (stage 35).
(A,B (enlarged, different embryo)) Each of the neural crest cell migratory routes are intensely stained with anti-FN.
(C,D (enlarged)) Anti-TN stains only areas in the medial portion of the embryo, i.e. the dorsal fin ECM, the medial surface of the somites and spaces around the neural tube, notochord and dorsal aorta. df, dorsal fin; som, somites; nt, neural tube; not, notochord; da, dorsal aorta; ms, myosepta; epi, epidermis. Bars, A,C: 200 \mu m; B,D: 100 \mu m.
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Fig. 8. Anti-tenascin and anti-fibronectin staining of transverse sections through the tail of stage 40 Xenopus larvae.
(A,B (enlarged)) With anti-TN an intense staining reaction is observed around the neural tube, notochord and throughout the ECM including intersomitic furrows and spaces around notochord and dorsal aorta. (C) Anti-FN stains the ECM surrounding the notochord intensely, but elsewhere the ECM is stained relatively faintly. nt, neural tube; not, notochord; ao, aorta; df and vf, dorsal and ventral fin. Bars, A: 100 μm; B, C: 50 μm.

Although the timing of appearance and the distribution of FN does not correlate well with the timing and pathways of amphibian neural crest cell migration, anti-FN staining is less intense and has a more restricted distribution at later stages in development when embryonic cell migrations have largely ceased.

The ubiquitous distribution of fibronectin suggests that some other component or components of the ECM may be responsible for directing the precise pathways of neural crest cell migration. One possible component is tenascin, a large ECM glycoprotein (Chiquet-Ehrismann et al. 1986) that has been shown to be present in the neural crest migratory pathways of avian and mammalian embryos (Tucker et al. 1987; Mackie et al. 1988). Tenascin, which was previously known as chick myotendinous antigen (Chiquet & Fambrough, 1984), is probably the same molecule referred to by others as cytotactin (Grumet et al. 1985), J1 (Kruse et al. 1985) or GMEM (Bourdon et al. 1985). As in the quail (Crossin et al. 1986; Mackie et al. 1988), TN is absent or has a very limited distribution in the trunk of amphibian embryos before neural crest cell migration. It is present in the embryo in an anterior-to-posterior gradient, roughly corresponding to the anterior-to-posterior wave of neural crest cell migration. Unlike FN, the distribution of TN is confined to the dorsal part of the embryo and in the dorsal and ventral neural crest pathways, as was shown previously (Mackie et al. 1988). During the late stages of migration, i.e. when pigment cell patterns are forming, TN has a broader distribution than FN. In the tails of stage-40 X. laevis larvae, TN is found between the muscle cells of the myotomes where neural-crest-derived pigment cells travel en route to the dermis (Tucker, 1986).

We were interested in determining if TN was found in the lateral neural crest cell migratory pathway of the dark axolotl (i.e. between the somites and the ectoderm) where, in contrast to the situation in X. laevis, the pigment cell precursors migrate (Vogel & Model, 1977; Löfberg et al. 1980; Keller & Spieth, 1984; Epperlein & Löfberg, 1984). According to the present data, the staining pattern with anti-TN was,
Fig. 9. Cultures of axolotl neural crest cells on fibronectin- and tenascin-coated substrata. (A) Neural crest cells spread from neural fold cultures onto FN-coated substrata, forming a sheet of cells (28 h). (B) Parallel neural fold culture (28 h); crest cells have not yet spread onto TN-coated substrata. (C,D) 3 days after explantation, neural crest cells have spread onto both FN- and TN-coated substrata, though the cells on FN are more flattened than the cells on TN. Bar, A–D: 100 μm.

however, strikingly similar between the axolotl and *X. laevis* during or just after neural crest cell migration: the staining was concentrated medially, in the ventral pathway, and was absent or very faint laterally. Thus the differences in pathways of migration of pigment cell precursors in *X. laevis* and the axolotl cannot be ascribed to differences in the distribution of TN. In addition to FN and TN, the distribution of a variety of other ECM components (e.g. hyaluronate and CSPG) should be investigated in detail to achieve a more comprehensive knowledge of the regulation of neural crest cell migration and localization in amphibian embryos.

The results of the culture experiments show that amphibian neural crest cells spread much earlier on FN- than on TN-coated substrata. After several days in culture, neural crest cells on FN are more flattened than the cells growing on TN. Similar results were obtained earlier with quail neural crest cells (Mackie et al. 1988). Though the initial spreading of the cells from the explants is delayed on TN-coated substrata, after 3 days the extent of migration is similar in cultures on TN and FN. We cannot rule out the possibility that after several days in culture the tenascin coating is modified in some manner, either through degradation or by substances secreted by the explant, that make it more adhesive to the migrating cells.

As in the embryos of birds and mammals, fibronectin and tenascin are present in the embryonic ECM that is used by neural crest cells as a migratory substratum in amphibian embryos. Fibronectin appears in the amphibian ECM before the migration of neural crest cells begins, whereas TN appears at the onset of migration and becomes more abundant and more widely distributed as morphogenesis proceeds. The distribution of TN is more closely correlated with the onset of migration and the pathways of neural crest cell migration than FN: TN appears in an anterior-to-posterior wave at a time corresponding to the anterior-to-posterior appearance of the neural crest, and it is restricted to the dorsal portion of the embryo, as are most of the derivatives of the neural crest. Nevertheless, we have shown that TN alone
cannot account for the migration of pigment cells and their precursors beneath the ectoderm in the axolotl. Although interactions between TN and FN, the precise nature of which is not yet understood, are likely to be involved in the initiation of migration and the ventral migration of amphibian neural crest cells, the lateral migration of pigment cells and their precursors are probably directed by other combinations of ECM materials.

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References


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